October 1, 1956

Dear Herman:

Your letter and note this AM reminded me to take care of commenting on Murahashi's ms. I agree that this ought to be published at the present stage of the work, and in very nearly its present form. I have made some comments on the ms. (in red pencil) which I summarize here. The ms. has been forwarded to Dr. Morse, who should return it to you promptly.

W-3178 is Gal_8^- and can be cited as such. (It was however, prepared by crossing with a Gal+ to secure a Gal_8^- prototroph, and its genetic background is therefore less certain than for the members of the W-309x series. For that reason, I rather thought to send another representative of this mutation as a check, but nothing seems to have come up so far that would warrant such extreme caution.) Present indications are that Gal2 and Gal8 belong to the same position-effect group, which begins to corroborate the cistron- enzyme correspondence, [in part: cf. W-3142!].

The exact references (as they now stand) to Morse et al. 1956a and b are on the enclosed sheets. b will be in the September issue, which is already overdue. I should be able to send you the page numbers in a couple of days. The same citation (i.e., sequence of authors) should be made for the reference 15.

Comments: TITLE "Gene and enzyme formation in galactose-negative mutants... The first two words add no sense, and I would delete them. Aside from rhetorical weakness, "gene and" is redundant to "in...mutants".

Abbreviations: Gal-1-P (G-1-P) is confusing. I would urge the following (definitions obvious):

PGlu PGal UPPGlu UPPGal UPPP (or UTP) . If nothing else, the transfer reactions are much more obvious in this form.

I agree reaction 3) should be called epimerase. Historically, waldenase has connoted (2) + (3).

p. 3 (Morse et al. Be more specific about the medium; I don't described any glycerol media in that reference. I think you should give some idea of the scale of the preparations: i.e., size of culture and weight of cells used. Are enzymes (1) and (2) adaptive?

p. 4 We have no direct evidence that the Gal loci are involved in biosynthesis of galactokinase unless the word is used in so broad a sense as to be almost meaningless. They are involved in the development of effective kinase activity.

Table 1. OK, except "W-3142: * no designation for this mutant, which is not linked in the same cluster as the others, has yet been published".

I don't understand table 2 at all. Are some headings missing—— oh, finally I see it. But I think it would be simpler to write explicitly something like the following:

Gal mutant(s)* Extract	Total counts incorporated
+	
1	
2	
3	
4	
W-3142	
1+2	
2+4	
1+4	
1+W3142	
2+W3142	
4+W-3142	

This can be set in double columns and be much more intelligible than you now ha it. If you want to keep the present general form, I would suggest alternatively that you rotate the table 90°: I would omit the - signs (which are what really h confused me)

Gal....

+	1	2	3	4	W-3142	Activity
+						33.9
	+					0
		+			etc	0
			+			0
	+					37.6

How many significant figures the zeroes have? Should you write 0.0 or 0.00?

You do not state the effectiv sensitivity of this procedure, i.e., whether the mutants mus have less then 1% or .1% or w (if any) of the amount of enz compared to +.

We very much appreciate the conservative way in which the genetic issues are stated here.

Now to your "crazy ideas". First of all, they are not the least bit crazy. The problem is whether the trans-heterogenote has a fully negative genotype, or one intermediate between the Gal- and the Gal+. We have very strong suspicions that the latter is the case for some heterogenotic combinations, but the problem is not necessarily the sensitivity of the methods. Also Pontecorvo has reported one definite case of intermediate position effect in Aspergillus.

We have in fact been wondering when it would be an appropriate time to needle you into making some quantitative enzymatic measurements on heterogenotes, both cis and trans, for comparison with the parent mutants and Gal+ (It is equally important to learn whether the cis- and the effective trans-heterogenotes are as efficient as the wild type). I don't think the problem is so much the sensitivity of the test as it is the difficulty of obtaining absolute genetic purity of a culture. Any heterogenote is constantly undergoing crossing-over and segregation, and therefore will contain some Gal+ recombinants. It should be possible to keep these at a level of, say 0.1 to 1.0% with careful technique, but the microbiological control will have to accompany the enzymology on each sample. For this reason, I thought we should wait on this problem at least until we had the full quantitative data on the existing cultures, as I am hoping to hear from Kiyoshi. If the quantity of cells involved are not too large, we might be able to handle them here. Alternatively, with labelled galactose it might simply be possible to determine how much label is incorporated by cells growing in its presence, and possibly this would require smaller amounts than would otherwise be necessary.

If your present assay methods, of modifications of them, can pick up 0.1 to 1% of the Gal+ activity, I don't believe they constitute a limiting factor at the present time.

Now your idea about using phage growth as an index of metabolic activity is not a bad one (though incorporation of C*Gal should be at least as sensitive would you have any trouble picking up the incorporation of even 1/1000 of the bacterial mass of C*?— compare this to the indicated 20% growth increment equivalent for phage). But it is not entirely new. Benzer*did a very nice study on lactase adaptation by this sort of technique, but using T2 or another phage, There would be real advantages to using a proper virulent phage. lambda is not likely to induce properly when its nutrition is suboptimal. A phage-plating method might be useful if the overall activity of a trans-heterogenote is such that we have to decide on its homogeneity in all the cells. But my own hunch is that it will be higher (at least in some cases) so that direct tests will be feasible. Alternatively, we may be able to develop some genetic tricks to prevent segregation.

*BBActa 11:383

I will be in Washington in a few weeks for an NSF panel. Could I see you the evening of Oct. 19? If I don't hear from you more definitely, I will call you during the lunch hour, or will wait for your call (either c/o Dr. George Lefevre, at NSF, or at the Lafayette Hotel.

Yours sincerely,

Joshua Lederberg